THE EFFECTS OF CERTAIN TYROSINE DERIVATIVES ON MAZE PERFORMANCE AND ACTIVITY LEVEL OF THE WHITE RAT

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By

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TABLE OF CONTENTS

Page

ACKNOWLEDGMENT ii

LIST OF TABLES iv

LIST OF DIAGRAMS vi

CHAPTER

I. Introduction 1

II. Apparatus and Procedure 9

III. Results 25

IV. Discussion and Conclusions 64

V. Summary 71

APPENDIX 74

BIBLIOGRAPHY 75

BIOGRAPHICAL SKETCH 78

LIST OF TABLES

Table Page

1. Weight Means and Standard Deviations of 41 Animals During Pre-experimental Period - Experiment II 19

2. Weight Means and Standard Deviations of Assigned Groups on Eighth Day of Pre-experimental Period - Experiment II 20

3. Maze Running Time in Seconds - Lashley Maze III - Experiment I 27

4. Maze Running Errors - Lashley Maze III - Experiment I. 28

5. Means of Groups with Respect to Time in Seconds - Lashley Maze III - Experiment I 29

6. Means of Groups with Respect to Errors - Lashley Maze III - Experiment I 30

7. Maze Running Time in Seconds - Lashley Maze III - Experiment II 32

8. Maze Running Errors - Lashley Maze III - Experiment II 36

9. Comparison of Groups with Respect to Time in Seconds - Lashley Maze III - Experiment II 40

10. Comparison of Groups with Respect to Errors - Lashley Maze III - Experiment II 41

11. Trials to Mastery - Lashley Maze III - Experiment II . 43

12. Comparison of Groups with Respect to Trials to Mastery - Lashley Maze III - Experiment II 44

13. Activity Wheel Revolutions Per 12-Hour Period - Spaeth Activity Wheel - Experiment II 46

14. Comparison of Groups with Respect to Activity Level - Spaeth Activity Wheel - Experiment II 47

15. Maze Running Time in Seconds - Elevated Stone Multiple T Maze - Experiment II 48

Table Page

16. Maze Running Errors - Elevated Stone Multiple T Maze - Experiment II 53

17. Comparison of Groups with Respect to Time in Seconds - Elevated Stone Multiple T Maze - Experiment II 58

18. Comparison of Groups with Respect to Errors - Elevated Stone Multiple T Maze - Experiment II 59

19. Trials to Mastery - Elevated Stone Multiple T Maze - Experiment II 61

20. Comparison of Groups with Respect to Trials to Mastery - Elevated Stone Multiple T Maze - Experiment II 62

21. Comparison of Groups with Respect to Weights at Completion of Experiment II 63

LIST OF DIAGRAMS

Diagram Page

I. Changes Following Conversion of Phenylalanine . . 6

II. Lashley Maze III 10

III. Elevated Stone Multiple T Maze 11

IV. Spaeth Activity Wheel 12

CHAPTER I

INTRODUCTION

The purpose of this study is to measure the effects of deprivation of phenylalanine and hence the lack of certain tyrosine derivatives, hydroxyphenylpyruvic acid and thyroxine, on certain psychological functions. The hypothesis to be investigated is that learning ability and/or activity level will be adversely affected by such deprivation.

The following reasoning was basic in arriving at this hypothesis. In 1934, Fölling (8) discovered that eight out of 830 mental defectives had unhydroxylated PPA in their urine, a condition referred to since that time as phenylpyruvic oligophrenia. Much is now known of the biochemistry underlying this syndrome. It is known that phenylalanine may be ineffectively converted into proteins (22) in the case of the mental defective with phenylpyruvic oligophrenia (14). It has been shown that this error of metabolism lies in the inability of the body to induce the hydroxylation of phenylalanine (3, 4, 6). The following facts complete the logic. In order for the amino acids to be utilized with maintenance of normal metabolic functions, control of the operation of the amino acid cycle must be provided (17). Tyrosine and hydroxyphenylpyruvic acid (hereafter referred to as OHPPA with PPA referring to phenylpyruvic acid in the abnormal unhydroxylated form) contribute to this balance in normal subjects (17, 19). Normal individuals can convert phenylalanine, an essential amino acid, into OHPPA and then oxidize this OHPPA via homogentisic acid to carbon dioxide and water. In the case of phenylpyruvic oligophrenia, however, the metabolic process is apparently blocked after phenylalanine is converted to PPA which fails to be hydroxylated by the addition of an -OH group. Large amounts of this unhydroxylated acid accumulate and must be excreted (17). This excess of PPA, before excretion, has theoretically "inhibited" the synthesis of acetylcholine which is believed to be fundamental in nerve conduction (20) and therefore may be regarded as indirectly related to psychological phenomena which are assumed to depend on neural processes--for example, learning and/or activity level. To be more specific, the crucial factor is choline acetylase, an enzyme responsible for rebuilding acetylcholine, which may be inhibited by the oxidation products of some amino acids, among which is PPA, an inhibitory product (17).

It is the belief of the present author that besides the inhibitory effects of PPA when in excess, too little OHPPA, brought about by depleting of phenylalanine, also interferes with protein metabolism and may result in inadequate neural functioning and correlated learning disability and/or decreased activity level. More precisely, if there is a deficiency of pyruvic acid, there cannot be an adequate synthesis into amino acids from pyruvic acid (which is necessary for normal carbohydrate metabolism, underlying nervous system functions) because reactions are unbalanced (15, 17, 19).

The deficiency within these complex metabolic processes could make the central and/or the peripheral nervous system function defectively. If so, this should be reflected in learning performance and/or activity level, respectively. It would seem logical to expect the organism deprived of phenylalanine to have a lowered basal metabolic rate generally and hence both inferior learning performance and lowered activity level, paralleling the sluggishness of the operation of the central and peripheral nervous systems. Then there is a further relation between these two psychological phenomena that bears noting—a minimum activity level must be maintained in order for learning to take place, particularly in perceptual motor problems such as the mazes employed in the present study. Activity level measurements on phenylalanine depleted animals would serve as a check against conclusions about learning without taking a minimum level of activity into account—no learning could occur, for example, if the animal was too weak to run in a maze. The finding that activity level alone was affected by phenylalanine deprivation could be considered as suggestive of the involvement of acetylcholine solely in motor or peripheral nervous system functioning; it might also suggest that OHPPA, in a reversible reaction, was being converted back into tyrosine and making much needed thyroxine available by this alternate pathway (See Diagram I).

Generally speaking, this experiment is an attempt to delineate one component of the biochemistry of learning and the related level of functioning intelligence and will leave to the biochemist such relationships among enzymes, acetylcholine, and OHPPA as may be indicated by further research.

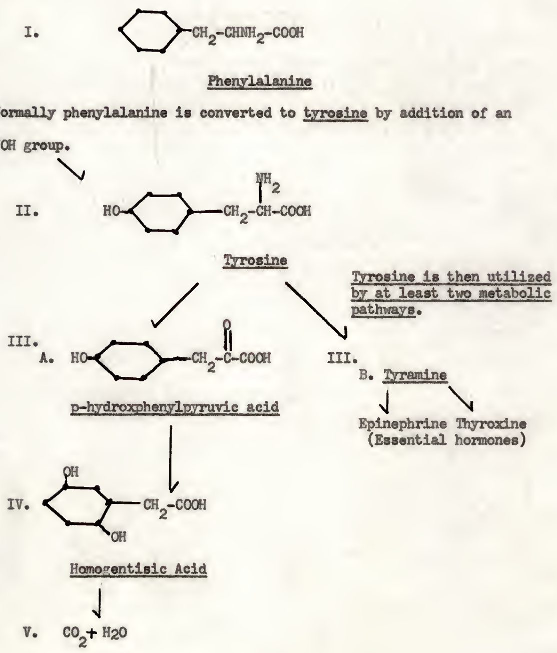
No studies of a psychological nature were found by this author in the literature on phenylalanine and its tyrosine derivatives. Some experimentation on phenylalanine and OHPPA of an exclusively physiological nature, however, has been carried out and will be reviewed as to the effects of phenylalanine on growth (1, 2, 5, 26, 28, 29) and on blood (13). The study of blood changes (13) seems to be the only investigation wherein OHPPA, or the hydroxylated form, was administered, but even here the dependent variable was analysis of blood content, a physiological matter. These studies indicate that phenylalanine, in proper form and amount, is essential for growth of living cells, but some of its derivatives such as PPA in excess, because of its decomposition products and/or competition with other metabolites necessary for the operation of enzyme systems, inhibit growth through resulting toxicity. This is particularly interesting since a parallel hypothesis—namely, inhibition from decomposition products—is generally accepted in explanation of some kinds of nervous system sluggishness and possibly inferior learning. Examples are cretinism and phenylpyruvic oligophrenia. It is this series of ideas which suggested to this writer that too little of the metabolite, OHPPA, might also interfere with protein metabolism and possibly be at the base of some varieties of mental defectiveness.

Before presenting a hypothesis and describing an experimental design for testing it, certain chemical relationships following the ingestion of phenylalanine and the changes of its derivatives in proper sequence need to be reviewed. Diagram I summarizes these facts as they have been accumulated from a synthesis of chemical research.

After ingesting phenylalanine, the normal organism converts it into tyrosine by the addition of -OH group. In turn this is converted into OHPPA and tyramine, the former resulting in homogentisic acid which is finally oxidized to carbon dioxide and water. Tyramine is converted into two hormones, epinephrine and thyroxine. The importance of thyroxine in modifying the effects of early cretinism, a pathological condition, the typical characteristics of which include mental defectiveness, has been recorded (10). It is, therefore, necessary to take account of the role of thyroxine and to control for its effects as has been done in the present study. It will remain for other investigations to manipulate the other metabolites in order to ascertain their effects on psychological

CHANGES FOLLOWING CONVERSION OF PHENYLALANINE

The only source of PPA or OHPPA is phenylalanine in the the diet.



Phenylalanine

Normally phenylalanine is converted to tyrosine by addition of an ⁻OH group.

Tyrosine

Tyrosine is then utilized by at least two metabolic pathways.

p-hydroxphenylpyruvic acid

Tyramine

Epinephrine Thyroxine
(Essential hormones)

Homogentisic Acid

Diagram I.

behavior.

Other investigations which confirm and give more detailed insight into the changes following phenylalanine conversion are found in studies by Moss and Schoenheimer (18); Painter and Zilva (21); Hall, Sydenstricker, and Rawls (11); Levy (16); Sanadi and Greenberg (24); Wooley (27); Nasset and Silciano (19); and Sealock, Perkinson, and Basinski (25).

This study, unlike the biochemical investigations referred to above, is concerned primarily with the effects of phenylalanine as they are related to psychological functions. As has been stated above, no studies of this nature were found in the literature. This situation is probably due to the technical problem in administering OHPPA as a variable, when it seemed that only the organism itself could hydroxylate it. Dr. E. W. Dennis and his staff at Sterling-Winthrop Research Institute provided this writer with synthetically hydroxylated phenylpyruvic acid (OHPPA), thereby making it possible to ascertain the effects of phenylalanine deprivation on learning performance and activity level, not only on a group of animals depleted of phenylalanine, but also on another such group with supplemented OHPPA administered orally.

A study of this kind appears timely in view of current research and biochemical theory (3, 4, 23) which indicate that enzyme failure is becoming increasingly important in understanding both physiological and psychological malfunctioning.

If the hypothesis is sound—that phenylalanine deprivation adversely affects learning ability—then it may be expected that Norway albino rats with diets depleted of phenylalanine will exhibit significantly different time and error scores and perhaps activity level in learning the Lashley Maze III, the elevated Stone Multiple T Maze, and running in the Spaeth Activity Wheel as compared with a normal group serving as a control and an experimental group receiving supplementary OHPPA. It is further expected that this last group of rats given supplementary OHPPA would not differ significantly in time and error scores nor in activity level from the normal diet control group.

CHAPTER II

APPARATUS AND PROCEDURE

Apparatus

Several different pieces of apparatus, designed to measure psychological functioning, were utilized in the two experiments of this study. These included the most difficult of the Lashley Mazes, an elevated form of the Stone Multiple T Maze, and the Spaeth Activity Wheel. While one Lashley Maze III was sufficient in Experiment I, two identical such mazes were used in Experiment II, making it possible to run two animals at the same time. Also in Experiment II the more difficult fourteen-cul Stone Maze was introduced as an additional dependent variable in the quest for a more sensitive measure of learning differences. Eight Spaeth Activity Wheels yielded measures of activity level differences in the same experiment. Diagrams II, III, and IV give the floor plans of the two mazes and a schematic representation of the activity wheel.

Uniform lighting at an approximate twelve-foot-candle level was obtained by placing the Lashley Mazes equidistant from the perimeter of a rectangular area of four ceiling lights. The Stone Maze was lighted by two suspended 150-Watt lamps. Temperature control was attained by selection of hours in an experimental day when

LASHLEY MAZE III

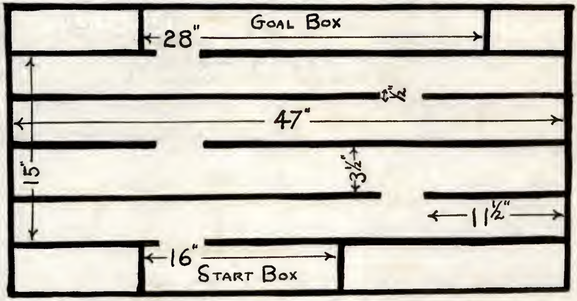


Diagram II

ELEVATED STONE MULTIPLE T MAZE

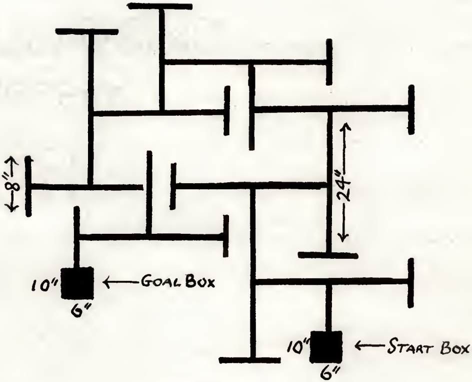


Diagram III

SPAETH ACTIVITY WHEEL

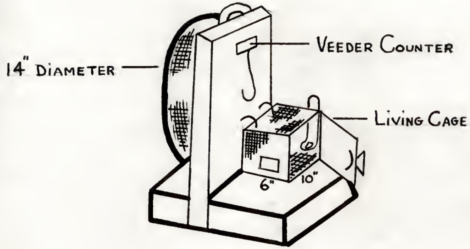


Diagram IV

a minimum of heat was expected—that is, from 5:00 a.m. to 8:00 a.m. and 6:00 p.m. to 9:00 p.m. During these times the temperature level varied only from 75° to 85° F. Counterbalancing running order of groups by taking all possible combinations of order provided the same mean temperature during runs for all groups.

Each animal was housed in a separate hardware cloth cage. Individual casters of heavy glass, which contained a specific amount of appropriate diet calculated daily on the basis of body weight, decreased food spillage, allowing for more accuracy of diet control. Individual water bottles with glass tubing provided a continual supply of distilled water.

Procedure

Two studies known as Experiment I and Experiment II were carried out and will be described separately.

Primarily, Experiment I, the preliminary study, was an evaluation of the experimental efficiency of the independent variables—namely, the five diet treatments. This was necessary due to the unknown potency of synthetically hydroxylated PPA. More specifically, there was some question as to whether or not oral administration of OHPPA would allow for sufficient organic absorption, since OHPPA is characteristically subject to rapid degeneration before reaching the point of tissue utilization in the peritoneum (7). Also certain interaction effects of thyroxine and OHPPA had to be ascertained before these two metabolites could be manipulated as separate variables. Other technical details such as uniform lighting, temperature and weight control, caging, and feeding to avoid spillage needed to be worked out prior to the critical second experiment. Experiment II, then, developed as an extension and elaboration of Experiment I in a more final effort to determine the effects of certain tyrosine derivatives on both maze learning and activity level. A larger number of animals of the same age, sex, and strain were employed in the second experiment in ascertaining the effects of the three critical diet treatments found in Experiment I. Forty-one 60-day old male, Norway albino rats of Wistar strain, each weighing between eighty and ninety grams, served as subjects.

Prior to any experimentation, it was necessary to define chemically the independent variables--namely, the complete and incomplete diets to be used. The writer is again indebted to Dr. Dennis and his staff at Sterling-Winthrop Research Institute for defining a phenylalanine-deficient amino acid mixture, the basis of the incomplete diet (7). The following is a list of amino acids and the constituent parts necessary to make approximately one gram (i.e. 1023.76 mg.) of the amino acid mixture:

Amino Acid	Milligrams
L-Arginine (HCL)	232.26
L-Histidine	66.70
DL-Isoleucine	91.40
L-Leucine	63.40
L-Lycine (HCL . H_2O)	112.30
DL-Methionine	45.40
DL-Threopine	71.70
DL-Tryptophane	28.30
DL-Valine	107.80
L-Glutamic Acid	204.50

(63.9 mg. of phenylalanine would make mixture complete)

The following list of vitamins was added in these proportions to each hundred grams of diet, both complete and incomplete (with the exception of "Navitol", which was necessarily added daily to individual portions because of the instability of Vitamins A and D):

Vitamins	Milligrams
Thiamine hydrochloride	0.92
Pyridoxine HCL	0.92
Methyl napthoquinone	1.15
Riboflavine	1.84
Niacin amide	4.6
Calcium pantothenate	5.1
Tocopherol	4.6
"Navitol" 65,000 u. vit. A/gm 13,000 u. vit. D/gm	112.0
Choline chloride	115.0
Biotin	0.06
Folic acid	0.06
Inositol	23.0

Five diet conditions can now be distinguished as the separate treatments employed in Experiment I. The following components and their proportions to the hundred gram quantity define the five treatments:

Group I Incomplete Diet - no phenylalanine

	Grams
Amino acid mixture	4.3
Sucrose	65.5
Cottonseed oil	17.4
Salt mixture (Wesen)	4.5
Salt flour (filler)	5.8

Plus vitamins listed above

Group II	Incomplete Diet - no phenylalanine - with supplementary thyroxine (25 mg.) (Otherwise same as incomplete diet of Group I, page 15)
Group III	Incomplete Diet - no phenylalanine - with supplementary p-hydroxyphenylpyruvic acid (50 mg. or twice the normal minimum daily requirement) (Otherwise same as incomplete diet of Group I, page 15)
Group IV	Incomplete Diet - no phenylalanine - with both supplementary thyroxine and p-hydroxyphenylpyruvic acid (same portions as Group II and Group III, respectively) (Otherwise same as incomplete diet of Group I, page 15)
Group V	Complete Diet

	Grams
Dried whole egg	20.1
Sucrose	58.3
Cottonseed oil	8.75
Salt mixture (Wesen)	4.5
Salt flour (filler)	5.8

Plus vitamins listed on page 15

In Experiment I the subjects were fourteen albino rats of mixed sex, age, and strain. The following five groups were matched as to weight and were assigned to the five diet treatments listed on pages 15 and 16:

Group I - two animals
Group II - two animals
Group III - four animals
Group IV - three animals
Group V - three animals

The diet portions of both Experiment I and Experiment II were figured daily on the basis of body weight—that is, one gram of diet to every twenty grams of body weight. After eight days of feeding each animal his respective diet, the rats were introduced to the Lashley Maze III. Each rat was allowed to spend a maximum of fifteen minutes in the maze. If he had not successfully found his way to the goal box by that time, he was picked up and fed there for ten seconds. A second trial was given immediately, again allowing ten seconds in the goal box. The animals were then returned to their individual cages and fed the remaining diet portion for that day.

Records of paths were kept by paper and pencil tracings on a diagram of the maze for each run. Errors were calculated from these tracings of trial runs with each cul entrance counting as an error. Time was recorded by stopwatch from the time of leaving the starting box to the entry of the goal box. All rats received a total of twenty trials over a period of ten consecutive days. Estimates of differences between groups with respect to time and error scores were made on the basis of an inspectional comparison of group means.

For Experiment II three critical diet groups were chosen based on the results of Experiment I. These were incomplete, incomplete with supplementary OHPPA (two times the normal daily requirement) and complete. Experiment II involved some changes in procedure and experimental conditions. A larger group of animals, this time forty-one rats of the same age (sixty days), weight (eighty-five grams), and sex (males), were used. Separate housing was again provided.

During the first ten days a diet of dog checkers and distilled water was given to all animals. This pre-experimental period was necessary to allow the animals complete adjustment to the laboratory situation and to handling by the experimenter; but more important, it was an opportunity to match groups for weight and direction of weight change. Biochemists declare body weight and/or surface to be the most important factor to control in a nutritional study of this kind (7). Tables 1 and 2 are records of weights recorded during this period, and the three groups of animals were formed on the basis of these matched means and weight standard deviations.

After analyzing the results of Experiment I, the three most critical of the five diet treatments were selected for more

TABLE 1

WEIGHT MEANS AND STANDARD DEVIATIONS OF 41 ANIMALS DURING PRE-EXPERIMENTAL PERIOD EXPERIMENT II

Days	Mean (Grams)	Standard Deviation (Grams)
1	78.02	3.58
2	92.10	3.82
3	98.24	4.27
4	97.88	5.30
5	94.22	5.70
6	100.22	3.98

TABLE 2

WEIGHT MEANS AND STANDARD DEVIATIONS OF ASSIGNED GROUPS ON EIGHTH DAY OF PRE-EXPERIMENTAL PERIOD EXPERIMENT II

Group	Mean (Grams)	Standard Deviation (Grams)
I Incomplete	111.43	4.98
II OHPPA	111.36	6.22
III Complete	110.85	5.43

accurately controlled study in Experiment II. Reliability of data was increased by using a larger number of animals in each group. The following groups and diet treatments were employed in this part of the study:

Group I	Incomplete Diet - no phenylalanine (Same as incomplete diet defined on page 15)
Group II	Incomplete Diet - no phenylalanine - with supplementary p-hydroxyphenylpyruvic acid (50 mg. or twice the normal minimum daily requirement) (Otherwise same as incomplete diet defined on page 15)
Group III	Complete Diet (Same as complete diet defined on page 16)

On the tenth day which concluded the pre-experimental period, the animals were continued on distilled water and placed on their respective diets of between five and six grams daily according to the formula of fifty milligrams of food per kilogram of rat weight. Beginning with the twelfth day, the animals were fed for one minute daily in the goal box of the Lashley Maze III, after which they were allowed to finish eating in their own cages. This procedure was adopted in order to form the association between the goal box of the maze and the reception of food. On the seventeenth day, each rat was given his first run in the Lashley Maze III. This procedure was continued twice daily at the specified morning and evening times until each rat had reached the established criterion of two consecutive, errorless runs.

Two running sessions were chosen per day at a time best suited to render equal temperature for all runs. Each rat was given a run between 5:00 a.m. and 9:00 a.m. and another between 6:00 p.m. and 10:00 p.m. Order of groups was counterbalanced to cancel out the effects of early hours and lower temperatures in the morning and later hours and lower temperatures at night. Temperatures varied between 75° and 85° F. Following each run, the rat was allowed to eat for ten seconds in the goal box; after the evening run, he was replaced in his cage and allowed to eat his daily allotment of his respective diet (except in the case of the animals in activity wheels, which is to be described below). In this manner each rat received two runs per day until he reached the criterion of two consecutive, errorless runs. Fisher's _t_'s were calculated for trials to mastery. An analysis of time and error score differences of the groups was made in the same manner.

Eight rats, three from the complete and incomplete groups and two from the OHPPA group, were placed in separate Spaeth Activity Wheels after the evening maze run and left there from 7:00 p.m. to 7:00 a.m. Over a period of the first six days of maze running, each animal spent twelve hours in this apparatus in order to determine and compare activity level of the three groups. Daily rotation of groups in wheels served as a control against possible differences in friction or other back action of the equipment. Revolutions per rat were recorded daily from the Veeder counters on the apparatus. Another Fisher's t test was computed to test the significance of differences between groups.

After the rats of the three groups had reached the established criterion of the Lashley Maze III, they were then further tested on an elevated Stone Multiple T Maze. The same criterion of learning (two errorless, consecutive runs) applied. Ten minutes was set as a time limit for the animals beginning this second maze. More animals were introduced to this maze as they reached criterion on the Lashley Maze III. A limit of ten minutes was sufficient time for all but three animals in the three groups; a limit of fifteen minutes was allowed for the "slower learners" from the Lashley Maze III as they were introduced to this more difficult fourteen-cul maze.

The following time schedule summarizes the procedure employed with the rats from the beginning of Experiment II through the beginning trials on the Stone Multiple T Maze:

Day	Treatment and Feeding
1	Dog checkers and distilled water; stabilization-pre-experimental period.
10	Began two experimental diets, incomplete and OHPPA, and one control diet, complete.

Day	Treatment and Feeding
12	Association training by feeding in Lashley Maze III goal box (diet continued during entire period of Experiment II).
17	First runs in Lashley Maze III.
18-23	Activity wheel for twelve hours, taking a total of eight rats per day from the three groups.
21	Stone Maze trials (beginning with animals as they reached criterion of two consecutive, errorless runs in Lashley Maze III).

CHAPTER III

RESULTS

In Experiment I records of the five groups on the Lashley Maze III were analyzed by counting the number of culs-de-sac entered per trial, thereby providing error scores, and taking time per run per rat in seconds for time scores. All rats were given twenty runs each. Criterion of mastery was set at four out of five errorless runs in less than ten seconds. Experiment II, in addition to Lashley Maze III data (same as Experiment I except that all animals were run twice daily until they each reached a criterion of mastery of two consecutive, errorless runs*), yielded records on the three groups from activity wheels indicating the number of revolutions each rat rotated the wheel in a twelve-hour period. The final set of data obtained in Experiment II was time, error, and trials to mastery on an elevated version of the Stone Multiple T Maze.

Due to the small number of animals in Experiment I, the only statistical treatment of data was the computation of time and error score means for the five groups. Fisher's t test of the significance of differences was used, however, throughout Experiment II where the larger number of animals employed would justify this more refined analysis.

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*One rat (Incomplete - E) was removed after failure to reach criterion in thirty trials.

Experiment I produced the raw scores of time and errors for each group as seen in Tables 3 and 4, respectively. It is to be noted in Table 4 that three of the four OHPPA's in Group III reached the set criterion. The only other animal doing so was a member of the complete diet group. Of all groups, then, only the supplemental p-hydroxyphenylpyruvic acid diet group approached the set criterion of learning of four out of five errorless runs in less than ten seconds.

The means of the five groups as to time and errors can be seen in Tables 5 and 6, respectively. On the basis of these results, it is suggested that thyroxine, when added to the incomplete diet, in spite of halving the running time, did not appreciably change the accuracy of learning the Lashley Maze III as compared to the performance of the incomplete group. The addition of OHPPA, however, to the incomplete diet appears to reduce time and errors in running the Lashley Maze III over the performance of the group on the incomplete diet. When the mean of Group III, or the group with OHPPA added to the incomplete diet, was compared to mean of the complete diet group, it appeared by inspection that little difference existed between them as to time and error performance.

TABLE 3

MAZE RUNNING TIME IN SECONDS
LASHLEY MAZE III
EXPERIMENT I

	Group I Incomplete		Group II Thyroxine		Group III OHPPA				Group IV OHPPA-Thyroxine			Group V Complete		
Animal	A	B	A	B	A	B	C	D	A	B	C	A	B	C
Trial 1	239	620	900	900	205	900	900	509	801	718	90	60	881	235
2	900	127	900	900	80	900	120	85	145	95	247	187	15	22
3	360	309	600	574	140	155	484	90	183	225	200	900	315	156
4	650	81	900	40	120	152	234	67	216	37	39	900	23	30
5	900	160	670	273	96	83	149	76	46	65	140	108	220	111
6	900	104	164	95	16	20	149	23	115	31	31	163	18	21
7	900	98	589	29	13	27	52	20	219	171	24	289	44	21
8	900	178	191	45	19	35	31	10	212	53	10	105	12	8
9	900	31	150	21	9	10	11	19	122	50	29	111	9	8
10	900	94	57	37	13	14	11	6	28	25	7	29	10	7
11	900	40	92	15	10	8	6	7	65	31	10	69	7	7
12	900	84	60	11	13	10	8	5	37	15	8	22	4	4
13	900	30	48	8	9	4	4	5	23	12	4	68	4	6
14	900	137	55	31	5	5	4	6	80	36	7	9	9	4
15	212	12	58	8	6	3	4	5	5	11	5	25	4	4
16	45	62	31	6	9	3	4	5	68	16	12	16	5	3
17	900	12	14	7	9	3	3	5	10	7	4	24	6	4
18	900	36	33	11	4	3	4	4	36	10	3	6	5	3
19	900	30	22	6	5	3	3	3	8	7	5	10	4	3
20	900	18	19	17	3	3	4	4	11	4	5	7	6	3

TABLE 4

MAZE RUNNING ERRORS
LASHLEY MAZE III
EXPERIMENT I

	Group I Incomplete		Group II Thyroxine		Group III OHPPA				Group IV OHPPA-Thyroxine			Group V Complete		
Animal	A	B	A	B	A	B	C	D	A	B	C	A	B	C
Trial 1	28	39	30	30	9	34	43	21	7	65	55	4	35	16
2	54	12	25	33	5	20	30	9	17	11	8	10	0	1
3	35	44	17	30	20	8	28	7	19	22	12	35	26	14
4	23	13	40	5	10	10	15	6	6	5	14	12	1	6
5	36	7	47	28	3	4	19	8	11	6	6	2	20	7
6	5	2	15	6	2	1	13	2	2	4	7	6	5	6
7	13	8	22	5	0	3	6	0	2	17	15	3	2	2
8	11	2	8	5	2	5	2	0	0	8	7	7	6	0
9	25	1	15	5	0	1	3	3	5	7	17	13	2	0
10	8	8	8	7	1	2	2	0	0	2	5	4	1	0
11	34	4	12	3	1	0	2	0	1	5	8	11	1	2
12	28	4	8	1	2	1	2	0	1	2	4	2	0	0
13	17	3	7	0	1	0	0	0	0	2	3	11	0	1
14	12	9	9	4	0	1	0	0	1	4	6	0	2	1
15	22	0	13	1	0	0	1	0	0	1	1	3	1	0
16	7	8	7	1	1	0	0	1	3	4	5	2	1	0
17	41	0	4	1	1	0	0	0	0	1	8	4	1	0
18	38	7	9	2	0	0	0	0	0	2	11	0	0	0
19	23	3	5	0	0	0	0	0	0	1	0	0	0	0
20	10	3	3	5	0	0	0	0	0	0	4	0	0	0

TABLE 5

MEANS OF GROUPS WITH RESPECT TO TIME IN SECONDS
LASHLEY MAZE III
EXPERIMENT I

Group	N	M
I Incomplete	2	431.67
II Thyroxine	2	214.67
III OHPPA	4	78.30
IV OHPPA-Thyroxine	3	82.15
V Complete	3	89.48

TABLE 6

MEANS OF GROUPS WITH RESPECT TO ERRORS
LASHLEY MAZE III
EXPERIMENT I

Group	N	M
I Incomplete	2	16.18
II Thyroxine	2	11.90
III OHPPA	4	4.64
IV OHPPA-Thyroxine	3	7.33
V Complete	3	4.82

Again by inspection, when the mean performance of Group I, or the incomplete diet group, was compared with the mean performance of the group on the supplemental diet of OHPPA-thyroxine, larger differences for time and errors were discernible. By inspection of means the complete diet group was better than the incomplete group. These mean differences are more than suggestive of a real difference in learning performance between the group of animals getting an incomplete diet with nothing added and those with OHPPA and/or OHPPA-thyroxine.

Experiment II produced the raw time and error scores on the Lashley Maze III shown in Tables 7 and 8. The larger number of animals in each group provided a better control over individual differences and made a more refined statistical analysis of data possible. Using Fisher's t test, a comparison of differences between groups with respect to time and errors was made. Results appear in Tables 9 and 10. In addition to statistical procedure, as already mentioned, the latter tables are not comparable to Tables 5 and 6 of Experiment I for several reasons. First, the raw time and error data was treated differently in the two experiments. In Experiment I, the means were computed on the basis of each score for each animal for each trial. This rough estimation of mean differences was employed as a result of the small number of animals as has been explained. In Experiment II, with a larger number

TABLE 7

MAZE RUNNING TIME IN SECONDS - GROUP I - INCOMPLETE
LASHLEY MAZE III
EXPERIMENT II

Animal		A	B	C	D	E	F	G	H	I	J	K	L	M	N
Trial	1	125	202	200	165	126	122	300	240	327	146	157	900	249	339
	2	310	163	354	416	355	900	44	46	89	739	563	900	215	257
	3	81	21	200	78	540	900	91	30	17	54	32	900	591	233
	4	22	15	28	7	95	81	96	10	10	25	19	174	11	127
	5	35	17	13	8	211	23	85	6	27	116	34	900	199	111
	6	9	4	7	4	90	14	7	5	11	17	9	655	7	53
	7	64	48	6	6	126	16	12	8	59	146	41	597	42	80
	8	5	4	5	6	48	5	4	4	9	15	11	333	5	33
	9	21	4		3	46	5	4	3	17	18	7	120	5	35
	10	5			4	12				17	7	5	57		15
	11	19				74				21	15	9	42		22
	12	6				10				10	17	4	31		18
	13	73				13				25	17	6	28		9
	14	7				8				8	12		33		5
	15	7				12				19	12		24		
	16	5				7				7	5		13		
	17	8				8				11	9		70		
	18	5				10				10	6		12		
	19	8				14				10	13		18		
	20	4				11				4	6		11		

TABLE 7—Continued

GROUP I - INCOMPLETE—Continued

Animal	A	B	C	D	E*	F	G	H	I	J	K	L	M	N
Trial 21					47					11		32		
22					22					6		12		
23					14					8		20		
24					11					5		8		
25					14					79		15		
26					10					5		7		
27					12					5				
28					10									
29					15									
30					8									

*E removed after failure to reach criterion in thirty trials.

TABLE 7—Continued

GROUP II - OHPPA

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Trial 1	94	80	263	180	165	60	45	25	304	194	486	900	50	195
2	52	235	312	60	23	36	21	95	30	48	80	156	20	99
3	98	61	386	70	48	20	101	10	26	19	15	66	43	36
4	34	9	218	96	10	32	42	29	8	145	9	53	38	38
5	45	66	24	37	9	17	96	6	102	14	9	28	26	8
6	11	5	20	35	4	12	9	4	6	8	4	25	5	4
7	32	11	13	10	46	29	6	7	7	7	10	15	13	5
8	15		5	6	4	5	4	3	4	4	4	7	7	
9	27		15	5	6	11	72	6			5	8	6	
10	9		9	3	3	4	4				3	10	4	
11	15		19		6	6	4				5	19	4	
12			7			6						5		
13			3			6						5		
14			3			3								
15						4								
16						3								
17						3								

TABLE 7—Continued

GROUP III - COMPLETE

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M
Trial 1	143	217	370	215	80	12	35	103	215	25	900	275	346
2	43	35	42	35	86	24	192	60	75	108	89	14	135
3	21	32	49	19	21	9	25	42	33	54	169	6	127
4	22	9	18	11	82	4	26	124	14	20	180	3	108
5	34	5	13	47	23	12	20	72	8	12	21	7	38
6	14	3	4	8	7	7	6	68	5	7	11	8	62
7	12	4	4	5	9	4	12	92	9	5	13	8	25
8	7	2	13	6	5	3	8	55	3	4	10	4	25
9	12	3	4		8	10	9	13	4		45	7	129
10	5		4		6	3	5	12			7	4	22
11	7		4		5	3	5	9			9		53
12	8		4		6			6			7		17
13	29		3		4			8			9		13
14	8				7						6		7
15	4				10						7		13
16	5				4						6		6
17					5						15		6
18											5		4
19											6		5
20													4
21													5

TABLE 8

MAZE RUNNING ERRORS - GROUP I - INCOMPLETE
LASHLEY MAZE III
EXPERIMENT II

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Trial 1	7	17	16	15	14	9	21	26	28	6	12	44	14	21
2	24	18	28	32	39	54	9	10	16	45	45	62	10	18
3	7	3	16	11	73	58	13	6	4	2	6	41	31	23
4	4	2	5	3	18	17	17	3	11	3	9	13	2	13
5	4	2	2	2	38	4	17	1	5	11	6	44	17	5
6	2	0	2	1	19	4	2	1	3	3	2	32	2	8
7	5	7	0	1	33	6	4	1	9	10	6	12	4	5
8	0	0	0	1	19	0	0	0	1	4	2	23	0	4
9	3	0		0	14	0	0	0	5	2	0	5	0	4
10	1			0	4				5	0	1	3		2
11	2				17				4	1	1	3		1
12	1				6				2	3	0	3		1
13	7				5				3	2	0	2		0
14	1				5				1	4		5		0
15	1				4				2	2		2		
16	1				5				1	0		1		
17	2				4				2	1		4		
18	1				5				1	1		1		
19	0				5				0	2		1		
20	0				5				0	0		2		

TABLE 8—Continued

GROUP I - INCOMPLETE—Continued

Animal		A	B	C	D	E*	F	G	H	I	J	K	L	M	N
Trial	21					13					1		3		
	22					7					0		2		
	23					5					2		2		
	24					5					0		1		
	25					6					4		0		
	26					5					0		0		
	27					5					0				
	28					4									
	29					6									
	30					5									

*E removed after failure to reach criterion in thirty trials.

TABLE 8—Continued

GROUP II - OHPPA

Animal		A	B	C	D	E	F	G	H	I	J	K	L	M	N
Trial	1	4	6	15	11	12	7	5	7	20	20	38	48	9	34
	2	4	16	30	9	6	8	5	17	5	10	16	15	5	13
	3	8	11	31	5	11	4	11	1	4	4	5	8	12	5
	4	4	1	29	10	2	9	6	7	1	26	2	14	9	6
	5	6	8	4	4	1	4	11	0	7	4	2	8	11	1
	6	0	0	4	4	1	3	4	1	1	4	0	6	1	0
	7	3	0	3	1	7	6	0	1	0	0	1	1	4	0
	8	2		2	1	0	1	1	0	0	0	0	1	2	
	9	3		2	0	1	1	9	0			1	1	2	
	10	0		3	0	0	0	0				0	3	0	
	11	0		5		0	2	0				0	1	0	
	12			2			2						0		
	13			0			1						0		
	14			0			0								
	15						1								
	16						0								
	17						0								

TABLE 8—Continued

GROUP III - COMPLETE

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M
Trial 1	13	22	24	14	10	3	8	11	19	4	47	26	24
2	6	7	6	4	10	7	20	10	11	12	7	3	10
3	2	9	11	2	3	3	5	10	6	6	22	1	11
4	4	3	8	1	16	0	5	24	4	3	19	0	11
5	6	1	5	6	4	2	3	11	2	2	4	1	5
6	2	0	1	1	2	1	1	13	0	1	2	1	9
7	2	1	0	0	2	1	2	11	1	0	2	3	4
8	0	0	1	0	2	0	2	9	0	0	2	1	5
9	1	0	0		2	3	2	1	0		4	0	17
10	0		1		1	0	0	1			1	0	3
11	1		1		0	0	0	2			1		4
12	0		0		1			0			1		2
13	3		0		0			0			2		2
14	2				1						2		2
15	0				1						1		2
16	0				0						1		1
17					0						2		1
18											0		1
19											0		1
20													0
21													0

TABLE 9

COMPARISON OF GROUPS WITH RESPECT TO TIME IN SECONDS
LASHLEY MAZE III
EXPERIMENT II

Group	N	M	Mean Diff.	σ Diff.	*t*	*P*
I Incomplete	14	1395.64	(I-II) 872.64	386.98	2.26	.05
II OHPPA	14	523.00	(II-III) 13.00	146.11	.09	. . .
III Complete	13	510.00	(I-III) 885.64	403.66	2.19	.05

TABLE 10

COMPARISON OF GROUPS WITH RESPECT TO ERRORS
LASHLEY MAZE III
EXPERIMENT II

Group	N	M	Mean Diff.	σ Diff.	*t*	*P*
I Incomplete	14	123.64	(I-II) 65.22	28.15	2.32	.05
II OHPPA	14	58.43	(II-III) 1.57	11.92	.13	. . .
III Complete	13	56.85	(I-III) 66.79	29.62	2.25	.05

of animals, means, standard deviations, standard errors of the means, and *t*'s were computed on the basis of total time and total errors to mastery for each animal. Also the tables of Experiment I and II for time and errors are based on different criteria of mastery—that is, larger means for time and errors are to be expected when they are computed on the basis of twenty trials per animal than on a basis of a lesser number of trials as was required to reach the less rigid criterion of two consecutive, errorless runs as in Experiment II. The differences between the incomplete and OHPPA groups were significant at the .05 and .05 levels of confidence for time and errors, respectively. The complete and incomplete groups were also different to the .05 and .05 levels for time and errors, respectively. No significant differences were found between the complete and OHPPA groups for either time or errors.

Another measure of learning performance was taken into account in Experiment II—namely, the number of trials to mastery, with mastery being defined as two consecutive, errorless runs (See Table 11). A comparison of groups with respect to trials to mastery (Table 12) on the Lashley Maze III showed the only significant difference to be between the incomplete and the OHPPA groups. A *t* of 2.11 was significant at the .05 level of confidence. Neither the OHPPA and the complete nor the incomplete and complete groups proved to be significantly different from each other on the basis of Fisher's *t* test in this particular measure of learning performance.

The next variable analyzed as to group differences was the

TABLE 11

TRIALS TO MASTERY
LASHLEY MAZE III
EXPERIMENT II

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Group I	20	9	8	10	30*	9	9	9	21	27	13	26	9	14
Group II	11	7	14	10	11	17	11	9	8	8	11	13	11	7
Group III	16	9	13	8	17	11	11	13	9	8	19	10	21	. . .

*Rat E Incomplete was removed after failure to reach criterion in thirty trials.

TABLE 12

COMPARISON OF GROUPS WITH RESPECT TO TRIALS TO MASTERY
LASHLEY MAZE III
EXPERIMENT II

Group	N	M	Mean Diff.	σDiff.	*t*	*P*
I Incomplete	14	15.29	(I-II) 4.72	2.24	2.11	.05
II OHPPA	14	10.57	(III-II) 2.12	1.40	1.51	. . .
III Complete	13	12.69	(I-III) 2.60	2.47	1.05	. . .

number of activity wheel revolutions, generally accepted as a measure of activity level. The raw scores for wheel rotations for the three groups appear in Table 13. When the three groups were compared with respect to activity level with a Fisher's t test of significance, the supplementary OHPPA group was found to be significantly more active than the incomplete diet group (.001); likewise, the complete group was significantly more active than the incomplete group (.01). The complete and OHPPA groups were not found to be significantly different as to activity level (See Table 14).

A final set of results was obtained by taking time, errors, and trials to mastery of the three groups on an elevated version of the Stone Multiple T Maze. The raw data for time and errors can be seen in Tables 15 and 16, respectively. Comparisons of groups as to time and errors are reported in Tables 17 and 18, respectively. Fisher's t test of the three groups indicates the OHPPA and complete diet groups both to be significantly faster than the incomplete diet group. This time the levels of confidence were .10 and .05, respectively. No significant difference was found between the OHPPA and complete groups. With respect to errors, only the complete group was more accurate than the incomplete group, the difference being significant at the .01 level; no significant difference was found between the OHPPA and incomplete groups. The differences of the complete and OHPPA groups with respect to errors were again not found to be significant.

The number of trials to mastery for the three groups on the

TABLE 13

ACTIVITY WHEEL REVOLUTIONS PER 12-HOUR PERIOD
SPAETH ACTIVITY WHEEL
EXPERIMENT II

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Group I	2556	3115	1152	1332	10	405	1	131	631	366	128	1212	1093	205
Group II	2675	3621	1574	3219	4132	3275	7361	1764	1469	1932	6563	3688	6439	4978
Group III	3261	1120	3718	6504	855	5828	2724	1562	1475	2914	1541	5291	77	. . .

TABLE 14

COMPARISON OF GROUPS WITH RESPECT TO ACTIVITY LEVEL
SPAETH ACTIVITY WHEEL
EXPERIMENT II

Group	N	M	Mean Diff.	σ Diff.	*t*	*P*
I Incomplete	14	881.21	(II-I) 2882.36	576.95	5.00	.001
II OHPPA	14	3763.57	(II-III) 927.42	761.50	1.22	. . .
III Complete	13	2836.15	(III-I) 1954.94	601.27	3.25	.01

TABLE 15

MAZE RUNNING TIME IN SECONDS - GROUP I - INCOMPLETE
ELEVATED STONE MULTIPLE T MAZE
EXPERIMENT II

Animal		A	B	C	D	E	F	G	H	I	J	K	L	M	N
Trial	1	333	245	600	511	226	231	375	150	306	561	465	827	495	400
	2	113	355	398	600	180	161	202	115	120	205	293	900	315	150
	3	96	78	180	265	181	105	107	75	46	90	29	790	40	80
	4	40	130	305	101	106	61	92	85	32	81	75	900	50	52
	5	39	101	171	45	38	30	33	35	34	29	18	900	25	74
	6	15	118	103	15	63	18	24	41	20	121	55	220	95	19
	7	45	36	42	35	28	17	14	21	18	20	13	860	41	30
	8	21	118	43	13	86	30	24	25	13	44	13	91	34	20
	9	35	25	18	18	52	57	25	22	29	12		276	19	27
	10	16	34	53		57	18	25	15		56		37	45	15
	11	49	13	54		76	16	11	49		49		21	20	19
	12	47	33	18		53	38	38	20		30		27		12
	13	19	17	41		50	16	9	10				146		
	14	22	42	48		38	16	28	11				20		
	15	38	16	12		60	12	9	15				96		
	16	11	22	14		145	15	20	10				51		
	17	18	10	20		48	19	8	9				30		
	18	11		34		205	11	15					18		
	19			18		100	10	7					54		
	20			16		50		30					16		

TABLE 15—Continued

GROUP I - INCOMPLETE—Continued

Animal		A	B	C	D	E	F	G	H	I	J	K	L	M	N
Trial	21			9		50		17					15		
	22			18		54		15					10		
	23			10		40		9							
	24			13		31		9							
	25			15											
	26			12											
	27			13											
	28			21											
	29			12											
	30			13											

TABLE 15—Continued

GROUP II - OHPPA

Animal		A	B	C	D	E	F*	G	H	I	J	K	L	M	N
Trial	1	224	301	269	258	344		153	119	333	89	223	121	260	192
	2	180	72	222	101	59		145	100	187	94	69	274	57	204
	3	75	18	89	26	28		63	60	88	111	31	125	55	85
	4	61	20	19	17	15		30	45	34	32	30	181	69	58
	5	29	12	56	21	10		16	58	31	18	20	72	16	33
	6	24	15	24	13	13		25	17	14	21	19	35	30	24
	7	16	19	10	14			12	23	15	22	11	17	17	42
	8	84	13	20	10			16	17	14	12	58	42	20	27
	9	27	9	47	10			21	12	13	8	14	22	11	16
	10	19	12	15	10			25	15	14	12	30	38	17	20
	11			19				10	16	8		11	15	10	13
	12			14				18	11			21	20	11	13
	13			28				9	9			10	14	8	9
	14			22				17				25	50		13
	15			21				11				12	22		
	16			13				11				15	20		
	17			29								21	9		
	18			12								36	16		
	19			13								9	25		
	20			12								31	20		

TABLE 15—Continued

GROUP II - OHPPA—Continued

Animal		A	B	C	D	E	F*	G	H	I	J	K	L	M	N
Trial	21			14								12	20		
	22			9								17	30		
	23											11	9		
	24											15	21		
	25											11	13		
	26											21	12		
	27											16	10		
	28											15			
	29											13			
	30											9			

*This animal was eliminated from experimentation on the Stone Multiple T Maze due to persistent climbing and failure to leave start box.

TABLE 15—Continued

GROUP III - COMPLETE

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M
Trial 1	600	242	125	217	309	150	323	328	157	345	472	183	251
2	410	195	146	177	166	53	141	480	78	89	230	54	222
3	250	36	16	42	76	36	85	102	17	31	124	62	88
4	59	28	42	58	40	39	31	39	63	31	157	15	98
5	29	20	12	27	51	34	21	23	24	27	42	18	40
6	21	15	12	48	23	11	18	27	15	22	35	23	43
7	30	23		17	23	9	13	14	11	23	21	22	25
8	13	12		31	18		20	17	14	15	44	19	25
9	14	11		18			12		18	11	13	20	18
10		10		36			15		11		24	13	
11		9		29			12		11		9	15	
12				25			28		9		43	22	
13				12			15		12		15	22	
14							22		10		11	17	
15							15		11		12	28	
16							32		12			13	
17							16		8			17	
18							23						
19							13						
20							12						
21							15						
22							11						
23							10						

TABLE 16

MAZE RUNNING ERRORS - GROUP I - INCOMPLETE
ELEVATED STONE MULTIPLE T MAZE
EXPERIMENT II

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Trial 1	11	10	10	8	9	4	7	8	9	11	11	11	7	8
2	5	9	9	10	6	7	9	10	7	5	6	11	6	7
3	3	2	7	7	7	8	8	8	4	2	2	8	1	3
4	3	5	12	6	7	5	7	5	3	3	4	10	1	2
5	3	5	7	2	3	3	3	5	1	1	1	11	0	2
6	0	8	8	0	1	1	3	4	1	2	1	6	3	1
7	2	3	4	1	2	0	1	3	1	1	0	8	2	1
8	1	5	2	0	4	2	1	2	0	1	0	4	2	1
9	0	3	1	0	3	3	2	2	0	0		3	2	1
10	1	2	2		3	1	1	0		2		1	0	1
11	1	1	5		4	2	0	4		0		0	0	0
12	3	2	1		2	2	2	0		0		1		0
13	0	1	3		1	2	0	1				5		
14	1	1	4		1	0	2	0				1		
15	1	1	1		3	1	0	1				2		
16	1	0	0		7	0	1	0				3		
17	0	0	2		4	3	0	0				2		
18	0		2		10	0	2					1		
19			3		5	0	0					0		
20			1		4		1					1		

TABLE 16—Continued

GROUP I - INCOMPLETE—Continued

Animal		A	B	C	D	E	F	G	H	I	J	K	L	M	N
Trial	21			0		5		2					0		
	22			1		5		1					0		
	23			1		0		0							
	24			1		0		0							
	25			1											
	26			1											
	27			1											
	28			1											
	29			0											
	30			0											

TABLE 16—Continued

GROUP II - OHPPA

Animal		A	B	C	D	E	F*	G	H	I	J	K	L	M	N
Trial	1	7	11	9	8	8		4	7	9	6	8	8	6	8
	2	6	5	8	5	5		7	5	8	6	7	8	6	8
	3	5	0	6	2	2		6	5	5	7	3	8	6	6
	4	3	1	2	1	1		3	2	2	4	4	8	4	4
	5	1	1	2	2	0		1	4	2	3	3	5	1	4
	6	1	1	2	1	0		1	1	0	1	2	2	2	2
	7	1	3	0	0			2	3	1	2	0	1	3	3
	8	5	1	2	1			1	0	0	1	4	2	3	3
	9	0	0	3	0			2	1	2	0	1	2	0	2
	10	0	0	1	0			2	0	0	0	2	2	2	1
	11			2				0	1	0		0	1	1	1
	12			1				1	0			2	2	0	1
	13			1				0	0			1	1	0	0
	14			2				1				1	2		0
	15			1				0				1	2		
	16			1				0				1	2		
	17			2								1	1		
	18			2								3	1		
	19			0								0	2		
	20			1								2	3		

TABLE 16—Continued

GROUP II - OHPPA—Continued

Animal		A	B	C	D	E	F*	G	H	I	J	K	L	M	N
Trial	21			0								1	3		
	22			0								1	2		
	23											1	1		
	24											1	2		
	25											0	2		
	26											2	0		
	27											2	0		
	28											1			
	29											0			
	30											0			

*This animal was eliminated from experimentation on the Stone Multiple T Maze due to persistent climbing and failure to leave start box.

TABLE 16—Continued

GROUP III - COMPLETE

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M
Trial 1	7	7	6	8	11	9	8	8	7	7	8	5	5
2	7	7	7	7	7	4	8	7	5	5	11	5	9
3	5	4	0	2	7	4	7	3	2	3	5	4	5
4	3	2	2	2	1	2	1	0	4	3	4	1	5
5	1	2	0	2	4	2	1	1	2	1	4	1	2
6	1	1	0	1	2	0	1	1	1	2	1	3	0
7	2	1		0	0	0	1	0	2	2	3	1	1
8	0	0		1	0		2	0	1	0	2	1	0
9	0	1		0			1		2	0	0	1	0
10		0		1			1		0		1	1	
11		0		1			0		1		1	0	
12				0			1		0		1	2	
13				0			1		1		1	1	
14							2		0		0	1	
15							1		1		0	1	
16							2		0			0	
17							1		0			0	
18							1						
19							1						
20							0						
21							1						
22							0						
23							0						

TABLE 17

COMPARISON OF GROUPS WITH RESPECT TO TIME IN SECONDS
ELEVATED STONE MULTIPLE T MAZE
EXPERIMENT II

Group	N	M	Mean Diff.	σ Diff.	t	P
I Incomplete	14	1592.79	(I-II) 913.64	404.81	2.26	.05
II OHPPA	13	679.15	(III-II) 73.77	113.10	.65	. . .
III Complete	13	752.92	(I-III) 839.87	411.57	2.04	.10

TABLE 18

COMPARISON OF GROUPS WITH RESPECT TO ERRORS ELEVATED STONE MULTIPLE T MAZE EXPERIMENT II

Group	N	M	Mean Diff.	σ Diff.	t	P
I Incomplete	14	48.86	(I-II) 13.48	8.33	1.62	. . .
II OHPPA	13	35.38	(II-III) 8.07	4.80	1.68	. . .
III Complete	13	27.31	(I-III) 21.55	7.50	2.87	.01

elevated maze can be seen in Table 19. A Fisher's t test on the differences between the groups with respect to trials to mastery showed only the complete group to be significantly better than the incomplete diet group (.05). Again no significant difference was found between the complete and OHPPA groups (See Table 20).

Finally, a comparison of weight of the three groups was made at the end of the Experiment II. Table 21 contains the means, sigmas, mean differences, as well as the t's and probability levels. Only the OHPPA's were significantly different in weight from the other two groups (.001). No significant difference existed with respect to weight between the complete and incomplete groups.

TABLE 19

TRIALS TO MASTERY
ELEVATED STONE MULTIPLE T MAZE
EXPERIMENT II

Animal	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Group I	18	17	30	9	24	19	24	17	9	12	8	22	11	12
Group II	10	10	22	10	6	. .*	16	13	11	10	30	27	13	14
Group III	9	11	6	13	8	7	23	8	17	9	15	17	9	

*This animal was eliminated from experimentation on the Stone Multiple T Maze due to persistent climbing and failure to leave start box.

TABLE 20

COMPARISON OF GROUPS WITH RESPECT TO TRIALS TO MASTERY
ELEVATED STONE MULTIPLE T MAZE
EXPERIMENT II

Group	N	M	Mean Diff.	σDiff.	t	P
I Incomplete	14	16.57	(I-II) 1.80	2.68	.67	. . .
II OHPPA	13	14.77	(II-III) 3.08	2.44	1.26	. . .
III Complete	13	11.69	(I-III) 4.88	2.29	2.13	.05

TABLE 21

COMPARISON OF GROUPS WITH RESPECT TO WEIGHTS AT COMPLETION OF EXPERIMENT II

Group	N	M	Mean Diff.	σDiff.	*t*	*P*
I Incomplete	14	85.29	(I-II) 9.36	2.30	4.07	.001
II OHPPA	14	75.93	(III-II) 11.07	1.98	5.59	.001
III Complete	13	87.00	(III-I) 1.71	1.99	.86	. . .

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Discussion

On the basis of the results of this study, it seems reasonable to assume that hydroxylated phenylpyruvic acid, referred to throughout this paper as OHPPA, plays an important role in the nervous system functioning of the albino rat. At the present time, an explanation in a biochemical framework has not been attempted. In spite of the complexity of the physiology involved, however, the main hypothesis of this study has been found tenable--phenylalanine deprivation has been accompanied by an adverse effect on learning performance of the rats of Group I, the incomplete diet group, as compared to Group III, the control group of animals receiving the complete diet. Furthermore, the administration of one of the tyrosine derivatives, OHPPA, to Group II, otherwise ingesting this same incomplete diet, has been followed by learning behavior which was not found to be significantly different from the control group of animals receiving the complete diet.

The finding that the OHPPA group was not significantly different from the complete group on any of the measured learning variables or in activity level and was significantly superior to the incomplete group in most of the measured variables is offered as evidence of the involvement of this metabolite in nervous system functioning. Whether OHPPA itself plays a specific role independent of the tyrosine-tyramine-thyroxine sequence (See Diagram I) or whether it is converted back into tyrosine in a reversible reaction, which becomes a source of needed thyroxine (7), remains to be determined by biochemists. Some tentative evidence for the opposite theory under investigation regarding the importance of OHPPA has been found in this study. The fact that adding OHPPA to the incomplete diet was followed by this group of animals learning as well as the complete diet group suggests that OHPPA might be a critical tyrosine derivative for certain performances involving efficient functions of the nervous system providing the OHPPA is given at an optimal dosage. It is even possible that the OHPPA cycle may have been the reversible reaction set off by the administration of thyroxine to cretins (10) (See Diagram I). This possibility is suggested further by the finding that the means of the thyroxine group of Experiment I were similar to the means of the incomplete group in learning performance, and the OHPPA-thyroxine group was by inspection similar to the OHPPA group itself. Of course, the small number of animals upon which this conclusion is based would necessitate further replications before it could be offered as conclusive evidence.

The full interpretation of a critical experiment completed in collaboration with a biochemist might elucidate the above problem as to the role of OHPPA if tyrosine and tyramine are also given to phenylalanine-deficient animals. In the meantime, explanations of physiological and biochemical foundations remain speculative.

Regardless of answers to the above problem, it seems clear that OHPPA works incrementally, whether directly or indirectly, in nervous system functioning and in subsequent learning. Rather than argue about organic and physiological counterparts of observed psychological behavior or the phylogenetic similarities of rat and human metabolism, it is urged that biochemists experiment with OHPPA on phenylalanine-deficient human beings. If an optimal amount of OHPPA can be found through functionally designed research, perhaps using mental defectives as subjects, it might prove quite valuable as a first step in a therapeutic direction, regardless of how it works biochemically. In light of the kind of physical and psychological improvements rendered to cretins by the administration of thyroxine (10), it would seem especially worthwhile to explore parallel possibilities of OHPPA administration on human subjects.

Certain observations of the effects of OHPPA reported in these experiments indicate that following its administration some kind of compensatory learning is occurring. This is deduced from the comparative results of the incomplete and OHPPA groups' performance in the maze situations. The full implications of these findings cannot be known prior to similar experimentation on human subjects, but it seems logical to assume that improved learning capacity (at least of some kinds of mental defectives) can be expected following OHPPA administration.

Observations in this study indicate the superiority of intraperitoneal injections as opposed to oral administration if the former could be carried out efficiently. In the first place, intraperitoneal injections would allow for more efficient absorption and possibly more pronounced psychological effects than oral administration because of the rapid degeneration rate of OHPPA before reaching the tissues at the point of utilization (7). Secondly, a more accurate control on the amount of food ingested would be provided than with oral administration and consequent food loss through spillage. The injections were used on the rats in the first experiment of this study but were rejected because of a high susceptibility to slough after OHPPA injections and because of high incidence of organic damage while attempting a penetration of the skin and peritoneal wall. With additional equipment and competence, however, these may not be problems for research on the human subject.

In addition to the broad suggestions for joint biochemical-psychological research suggested in the above paragraphs, more specific answers of a psychological nature should be sought. First, there is the question of the permanence of the psychological effects of phenylalanine deprivation. A further study could be carried out wherein the three diet treatment groups were tested and retested on two alternate forms of a group of standardized learning tasks before and after returning these groups to a normal diet. Hebb and his students at McGill University have made such a measure available (12). If recovery of psychological behavior level occurs on this normal diet, the effects of phenylalanine deprivation could be considered temporary. Such a finding would be optimistic in the final effort to treat mental deficiency of the variety related to such metabolic errors resulting from either too much PPA or too little OHPPA. This recovery might indicate only temporary impairment to nervous system functioning. If, on the other hand, experimentation indicated the effects of such deprivation to be a permanent impairment of nervous system functioning, certain limits would be placed on the usefulness of OHPPA as a therapeutic device. It would also be interesting to compare the retention of the three groups employed here after being placed on a normal diet for a period of time. The writer believes an extensive investigation of permanence of effect justified on the basis of the findings in the series of studies reported here.

Conclusions

1. The hypothesis that deprivation of phenylalanine and hence OHPPA and thyroxine would adversely affect learning performance and/or activity level in the albino rat has been found tenable in

this study. The following findings are offered as evidence for this hypothesis:

a) When the incomplete diet group was compared to the complete diet group, scores on the Lashley Maze III, Stone Maze, and Spaeth Activity Wheel indicated the incomplete group inferior in learning performance and activity level to the complete group.

b) When OHPPA was added to the incomplete diet and the performance of this group on these mazes and in the activity wheel was compared to the incomplete diet group without this metabolite, the OHPPA group was in all cases, with the exception of errors and trials to mastery on the Stone Maze, significantly superior to the incomplete group.

c) When the OHPPA group performance on the mazes and in the activity wheels was compared to the complete diet group, there were no significant differences found between these two groups. It may be concluded, generally, that adding OHPPA to the incomplete diet was followed by performances similar to that of the control complete diet group.

2. A tentative conclusion may be drawn from Experiment I on the effect of thyroxine on learning performance on the Lashley Maze III. Due to the similarity of the means found between the thyroxine and incomplete groups and the further similarity of the means of the OHPPA-thyroxine group and the OHPPA group, it is a guarded conclusion that OHPPA and not thyroxine may be the critical

factor involved in nervous system functioning and related psychological behavior. It is to be noted that this is a qualified conclusion due to the small size of the samples and crude statistical evaluation employed in Experiment I.

3. The OHPPA group, matched for weight with the other groups at the beginning of the experiments, had lost weight by the end of the experiments so that the animals of this group were significantly lighter than either of the other two groups. No significant differences existed between the complete and incomplete groups with respect to weight.

4. In addition to such organic changes as white feces for the complete, cream-colored for the incomplete, and light brown for the OHPPA, indicating the diets were having concrete effects, definite behavioral changes were observed to occur in the three groups of animals—the incomplete group was consistently more aggressive, and even vicious at times, as compared with the more docile OHPPA and complete diet groups.

CHAPTER V

SUMMARY

Two experiments were carried out in this study to assess the effects of phenylalanine deprivation (and hence certain tyrosine derivatives—namely, OHPPA and thyroxine) on psychological functioning.

Fourteen albino rats were divided into five diet groups including incomplete, supplementary thyroxine, supplementary OHPPA, supplementary OHPPA and thyroxine, and complete and tested on the Lashley Maze III. By inspection of means the complete, OHPPA, and OHPPA-thyroxine groups appeared similar to each other and were faster and more accurate in learning time and errors than the incomplete diet group.

The second experiment employed forty-one albino rats matched for age, sex, weight, and strain. Three diet treatments, incomplete, OHPPA, and complete, were tested in the Lashley Maze III, Spaeth Activity Wheel, and on an elevated version of the Stone Multiple T Maze. In most cases the OHPPA and complete diet groups, though not significantly different from each other, were significantly better than the incomplete group both in time, error, and trials to mastery on the mazes, and in higher activity levels in the wheels.

OHPPA is concluded to be an effective variable in level of nervous system functioning as manifested in the learning of two mazes and in the differences in activity level.

Several suggestions for further research are made, particularly studies of the psychological effects of OHPPA injections on mental defectives paralleling the work previously done on cretins (10) with thyroxine.

APPENDIX

ROTATIONAL SCHEME FOR ACTIVITY WHEELS

Day	Group I Incomplete	Group II OHPPA	Group III Complete
18	A B C[a] (1-3)[b]	A B (4-5)	A B C (6-8)
19	D E F (6-8)	C D (1-2)	D E F (3-5)
20	G H I (4-6)	E F (7-8)	G H I (1-3)
21	J K L (1-3)	G H (4-5)	J K L (6-8)
22	M N (4-5)	I J (1-2)	N (3)
23		K L M N (3-6)	

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[a] Individual animals of each group designated by letters of the alphabet.

[b] Numbers in parentheses refer to activity wheels.

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BIOGRAPHICAL SKETCH

Elizabeth Hammond Faulk was born May 18, 1925, at Jacksonville, Florida. She graduated from Robert E. Lee High School there in 1943. She received her Bachelor of Arts Degree from Connecticut College, New London, Connecticut, in 1947 with a major in philosophy.

After teaching one year of elementary and one year of junior high school, she began graduate study, taking clinical courses at Columbia University and receiving her Master of Arts Degree in psychology from the University of Florida in 1950.

From that time until the fall of 1954, Miss Faulk taught psychology and directed the guidance and counseling program at the Jacksonville Junior College, except for the year 1952-1953 when she received a leave of absence to accept a doctoral fellowship at the University of Florida.

At the present time, she holds the position of psychologist for the Caddo Parish School Board, Shreveport, Louisiana. She is also currently teaching night courses in psychology at Centenary College of Louisiana.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Arts and Science and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

January 29, 1955

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